

AMENDMENTS TO THE SPECIFICATION

Please amend the application as indicated hereafter. It is believed that the following amendments and additions add no new matter to the present application.

In the Title:

Please replace the title with the following new title:

Methods For Preparing Yeast With Improved Biotin Productivity Using Integrating Plasmids Encoding Biotin Synthase

In the Abstract:

Please replace the pending Abstract with the newly-submitted abstract attached herewith on a separate sheet.

Methods for preparing yeast with improved biotin productivity using integrating plasmids encoding biotin synthase. ~~The invention discloses a yeast with high biotin productivity and the preparation thereof.~~ The yeast is transformed by an integrating plasmid, which includes a *Candida utilis* biotin synthase gene BIO2, an assistant DNA sequence to promote for the integration of the plasmid into the *C. utilis* a host genome, a promoter sequence, and a selection marker. Other embodiments include *Saccharomyces cerevisiae* integrating plasmids.

In the Specification: [Use ~~striketrough~~ for deleted matter and underlined for added matter.]

Please amend the paragraph starting on p. 3, line 11 as follows:

Most organisms require biotin to survive, and only a few organisms can synthesize biotin themselves. The conditions due to lack of biotin in human and animals are not common; however, under some hereditary diseases or dystrophy, the lack of biotin usually results in severe consequence. For example, babies that lack holocarboxylase synthase usually have symptoms such as vomiting or asthma from the first day they are born; babies that lack ~~biotindase~~ biotindase may have disorders with skin, eye, and urinary system when 2-3 years old. In the past 10 years, it has been found that biotin is associated with the problems of farm animals that

are bred on a large-scale. It is notable in poultry, for example, having fatty liver and kidney syndrome (FLKS), and acute death syndrome (ADS) in chicken. In 1977, it is also found that the cylopodia (crooked-foot disease) in indoor-bred pigs is associated with the lack of biotin. Therefore, the demand of biotin increases 15% per year, in which 60-80% of biotin is used as feed additives.

Please amend the sentence starting on p. 7, lines 15-17 with the following sentence:

In these plasmids, the BIO2 synthase gene selected from *S. cerevisiae* or *C. utilis* can express biotin synthase, in the latter of which the nucleotide sequence is set forth in SEQ ID NO: 1.

Please amend the paragraph starting on p. 9, line 25 with the following:

Example 1. Cloning of *S. cerevisiae* BIO2 Gene

The primers containing S all site for cloning were designed according to the BIO2 gene sequence of the *S. cerevisiae* Y266 strain as follows as SEQ ID NO: 2 and SEQ ID NO: 3, respectively:

Forward: 5'-GAAAGTCGACTCAAGATCTGTCGTAATA-3'; and

Reverse: 5'-CCGCAGTTAAATCG'ACAAGT'-3'.

Please amend the paragraph starting on p. 10, line 4 with the following:

Example 2. Cloning and Sequencing of *C. utilis* BIO2 Gene

Two conservative amino acid sequence regions were obtained when compared to the biotin synthase gene of five microorganisms belonging to *S. cerevisiae*, *E. coli*, and *Erwinia herbicola*. The degenerate primers were designed according to these conservative regions as follows as SEQ ID NO: 4 and SEQ ID NO: 5, respectively:

Forward: 5'-TGTNCNGARGAYTGAAANTATTG-3';

Reverse: 5'-GTRTCNANRTTGTG'GTTGTA-3',

wherein Y=T+C, and R=A+G'. About 0.3 kb of DNA fragment was obtained from *C. utilis* genome by PCR. This DNA fragment was used as a probe for screening the complementary sequences from *C. utilis* genomic library prepared by the inventor (using lambda EMBL3 as a vector).

Please amend the sentence starting on p. 14, line 24 as follows:

Besides 23 ng/ml biotin originally biotin originally present in YPD medium,
only 8.231.2 ng/ml biotin is produced by the wild type (WT) yeast.

Please amend the Table on p. 15, Table 1 as follows:

	Culture Broth				Cell Extract		
		YPD (ng/ml)	OD ₅₅₀ nm	YPD(CHY) (ng/ml)	YPD (ng/ml)	YPD(CHY) (ng/ml)	incubator
YPD		23					
WT	8.72	<u>2631.2</u>			5.3		
m9-101	9.05	860	8.04	660	66	63	Flask(50ml)
m9-102	8.75	760	2.28	714	86	7.3	Flask(50ml)
m9-103	5.12	920					Tube(3ml)
m9-104	8.40	1010					Tube(3ml)
m9-105	8.90	900					Tube(3ml)
m9-106	6.00	750					Tube(3ml)
m9-107	5.96	460					Tube(3ml)
m21-101	8.44	600	2.56	620	98	6	Flask(50ml)
m21-102	6.28	802	4.72	964	111	13	Flask(50ml)
m21-103	3.60	420					Tube(3ml)
m21-104	8.54	720					Tube(3ml)
m21-105	<u>8.38</u>	430					Tube(3ml)
m21-106	4.86	560					Tube(3ml)